Chromosome Numbers in the Fern Genus Anogramma, II.

JUDITH G. BAROUTSIS* and GERALD J. GASTONY**,[1]

In an earlier report on chromosome numbers in the genus *Anogramma* (Gastony & Baroutsis, 1975), three new counts were established and all known previous counts were summarized. At that time, a count of $n=26$ for *A. leptophylla* from Europe (Kurita, 1971) was overlooked. We wish now to acknowledge this count, to report new counts made for populations of *A. guatemalensis* and *A. leptophylla*, and to suggest an explanation for some of the variant counts previously reported for *A. leptophylla*.

The general techniques for chromosome preparations were those previously discussed (Gastony & Baroutsis, 1975). To maximize chromosome staining, however, a propionic-iron-haemotoxylin stain (Henderson and Lu, 1968; Rigby, 1973) was applied to fixed mitotic cells of *Anogramma* gametophytes. The stained chromosomes were visually enhanced by use of phase microscopy in analysis and photographic work.

To promote spreading and separation of mitotic cells during squashing, material was treated with one of two preparations: Glusulase (Endo Laboratories Inc., Garden City, NY), a commercially available enzyme mixture from the intestinal juice of the snail *Helix pomatia*, was applied full strength to gametophyte tissue for four hours (Fabergé, 1945); Driselase (Kyowa Hakka Kogyo Co., Tokyo, Japan), a fungal-produced enzyme mixture, was applied as a 10% (w/v) aqueous solution according to the Glusulase schedule. Both preparations were equally satisfactory for softening cell walls. The potential of this enzyme technique in working with gametophyte chromosomes has been more fully discussed by Gastony (1977).

Chromosome counts for *A. guatemalensis*, published here for the first time, are based on three unequivocal counts. The counts reported for *A. leptophylla*, however, are based only on the material illustrated in *Figs. 3, 4, 8*, and *9*.

Sources of spores cultured to provide living material of the taxa herein reported are: *A. guatemalensis*, *Gastony 1037*, Depto. Chimaltenango, Guatemala; *A. leptophylla*, 7 Oct 1972, *Mickel*, Edo. Oaxaca, Mexico; *A. leptophylla*, 20 Oct 1972, *Esterhuysen*, Cape Province, South Africa. Voucher specimens of the plants raised from spores are deposited at IND.

RESULTS AND DISCUSSION

Mitotic squashes from gametophytes of *A. guatemalensis* and *A. leptophylla* show a chromosome number of $n=29$ (*Figs. 1-7*). Chromosomes of both these species have a strong tendency to stick together, particularly at their ends. This stickiness, in conjunction with the form of two of the chromosomes as discussed

*Department of Biology, Augusta College, Augusta, GA 30904.
**Department of Biology, Indiana University, Bloomington, IN 47401.
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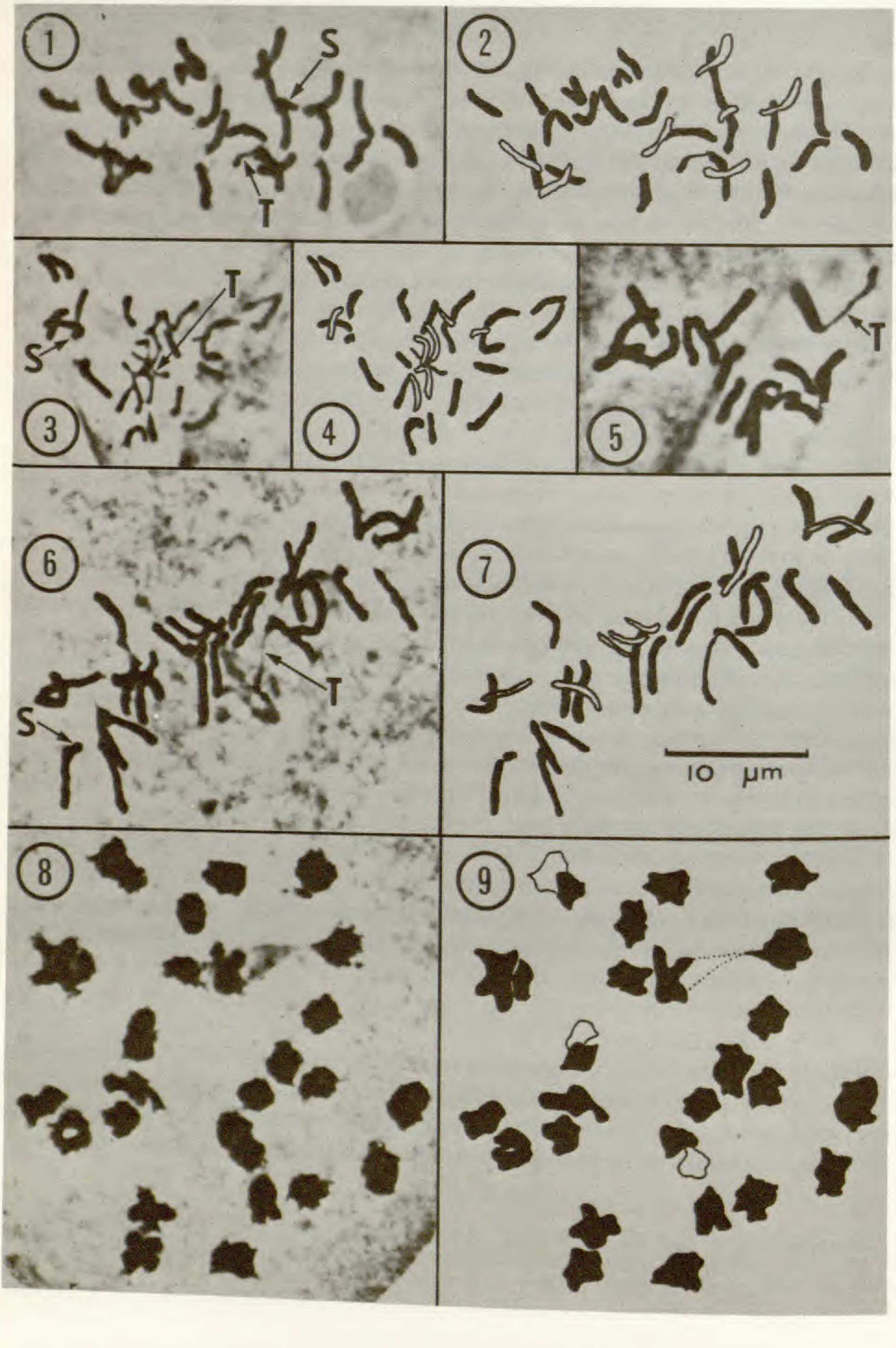
1
S
T
2
3
S
T
4
5
T
6
S
T
7
10 µm
8
9

below, may explain the variability in the counts that have been reported for Mexican *A. leptophylla* (Mickel et al., 1966) and in at least some of the other previously reported counts which are at variance with a base number of 29.

An analysis of chromosome morphology in the course of this work has proven useful in ascertaining the actual chromosome numbers present and in determining the source of variability often encountered in *Anogramma* squashes. From observing numerous cells, it is known that both *A. leptophylla* and *A. guatemalensis* have one very thin chromosome (*Figs. 1, 3, 5,* and *6*) and a short chromosome that frequently appears as a knob at the end of another chromosome (*Figs. 1, 3,* and *6*). Prior to squashing, and often in squashed preparations, the short chromosome looks like a satellite. After squashing, however, it often lies at right angles to the chromosome with which it is associated (*Figs. 3* and *6*) or across this chromosome (*Fig. 1*). The thin chromosome also seems always to be associated with another chromosome, but in several cases it has been found completely free. The reason for these chromosomal associations is unknown. In at least half (ca. 10) of the cells examined, one or both of these chromosomes is not evident, and when counted, these cells appear to have 27 or 28 chromosomes.

As in mitotic preparations, meiotic squashes also reveal a tendency for chromosomes to stick to one another. The resultant difficulties were noted earlier (Gastony & Baroutsis, 1975) and were experienced again in attempts to count *A. leptophylla* from South Africa. Only one clear meiotic count of $n=29$ has been obtained from this South African material thus far (*Figs. 8* and *9*). The conditions causing chromosomal clumping thus appear to be present in both meiotic and mitotic cells.

The similarity in chromosomal morphology and behavior in gametophyte cells of *A. guatemalensis* and *A. leptophylla* parallels other shared features, such as identical spore morphology, similar gametophyte development, and similar physiological response to growth conditions (Baroutsis, 1976). Altogether, this evidence supports Tryon's (1962, p. 75) suggestion that *A. guatemalensis* may be an infra-specific variant of *A. leptophylla*. Final taxonomic disposition, of course, will require comparative morphological studies of populations throughout the North and Central American ranges of these two species.

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FIGS. 1-9. Photographs and camera lucida clarifications of *Anogramma* chromosomes of specimens cited in the text. FIGS. 1-7. Mitotic figures from gametophyte cells. FIGS. 8-9. Meiotic figure from spore mother cell. T = thin chromosome, S = short chromosome. FIGS. 1-2. *A. guatemalensis*, $n=29$. FIGS. 3-4. *A. leptophylla*, Mexico, $n=29$. FIG. 5. *A. guatemalensis*, portion of a squash included to show thin chromosome, T. FIGS. 6-7. *A. guatemalensis*, $n=29$. FIGS. 8-9. *A. leptophylla*, South Africa, $n=29$.

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Curtis Eugene Delchamps (1925-1977)

Curtis Eugene Delchamps was born March 3, 1925, in New Orleans, La., but grew up in Mobile, Ala. He studied chemistry at the University of Alabama, Pennsylvania State University, and West Virginia University where he received the Ph.D. degree. While still a student at the University of Alabama, he married Earsie Ward, who was also a chemistry major. He started teaching chemistry at the University of Miami, Miami, Florida, in 1955, and continued there until his death September 12, 1977, from a heart attack.

Gene had a life-long interest in nature, especially wild flowers, and became interested in photography as a means of studying plants. When he first moved to Florida, he started to learn about the plant life there, both native and cultivated. He soon discovered that the local plant experts knew very little about the native ferns. Learning about them was a challenge to him, and he worked very hard on the group and soon became an authority in the field. He combined his plant expertise with his photographic skills, and became a popular lecturer on ferns.

He served for two terms as president of the Miami Men's Garden Club, and helped to bring national recognition to the group by sponsoring a successful candidate for the Johnny Appleseed Conservation award. He was first president of the South Florida Fern Society in Miami, and continued to serve on its Board of Directors until his death.

Perhaps Gene's greatest contribution to the world of ferns was his enthusiasm for learning and his eagerness to share his knowledge with others. This he did by organizing and conducting field trips, setting up study groups, giving numerous talks to garden clubs, and contributing educational articles for the club newsletters.

He is survived by his wife, Earsie, a daughter, Barbara, and a son, Charles-

Mrs. C. E. Delchamps, 18240 S.W. 248th St., Homestead, FL 33031.